A New Approach for Capillary Ion Chromatographic Separation of Carbohydrates and Amino Acids

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White Paper 70462

Executive Summary

Capillary-scale IC offers substantial benefits over conventional IC, including higher mass sensitivity, improved separation efficiency and/or speed, smaller sampling volumes, reduced eluent waste, and continuous system operation. Capillary IC platforms with on-line electrolytic eluent generation enable highly reproducible isocratic and gradient separations of target analytes with excellent resolution.

Capillary IC coupled with electrochemical detection is a practical and cost-effective approach for the analysis of carbohydrates and amino acids.

Key Words

RFIC, Capillary IC, Electrolytic eluent generation, Carbohydrates, Amino acids

Overview

Purpose: In this work, studies on a new platform to perform capillary-scale gradient separations of carbohydrates and amino acids using multi-component eluents generated electrolytically are carried out.

Methods: Capillary electrolytic KOH and MSA generator cartridges are used in tandem to generate eluents containing KOH and potassium methanesulfonate at low µL/min flow rates. The combined use of capillary electrolytic eluent generators provides high-fidelity gradient profiles for multi-component eluents without the need for mechanical proportioning.

Results: The prototype Reagent-Free™ Ion Chromatography (RFIC™) systems employing this platform have been used successfully to achieve capillary-scale gradient separations of sialic acids, inulin oligomers, and 17 amino acids with good chromatographic resolutions of target analytes.

Introduction

Capillary IC has gained increasing interests in recent years. Compared to conventional-scale IC systems, capillary IC systems offer several advantages. The capillary IC systems typically operate at 5 to 20 µL/min and thus the amount of eluent consumed is very small. The capillary IC systems have improved capability for continuous operation with minimal intervention. The use of a capillary column improves the separation efficiency and/or speed. The operation of capillary IC at low flow rates improves the system compatibility with mass spectrometers. Separation processes in the capillary format require a much smaller amount of sample and thus offer improved compatibility with applications where amount of sample is limited.

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The separations of carbohydrates and amino acids in ion chromatography are typically achieved using multi-component eluents at varying concentrations. In conventional-scale IC using 2 mm or 4 mm columns, the delivering of multi-component eluents in gradient separations is accomplished using a high-pressure pump fitted with a proportioning valve capable of proportioning up to four eluent components to vary the eluent composition in the gradient separation. The conventional-scale gradient pumps have delay volumes of about 1 mL associated with the pump heads and the proportioning valve. This delay volume is not compatible with capillary-scale IC separations since it corresponds to a delay time of 100 min if the separation is performed at 10 µL/min. To explore the benefits of capillary IC for determination of target analytes such as carbohydrates and amino acids where multi-component eluents at varying concentrations are required, we have carried out preliminary studies on a new platform to perform capillary-scale gradient separations using multi-component eluents. In this white paper, we will discuss the operation principle of this new platform and demonstrate its applications in the separations of carbohydrate and amino acids.

Experimental

All experiments were performed using a Thermo Scientific™ Dionex™ ICS-5000 capillary RFIC system equipped with an electrochemical detector. The Dionex ICS-5000 capillary RFIC system was controlled by Thermo Scientific™ Dionex™ Chromeleon™ 6.8 Chromatography Data System software.

A block diagram of key components in a prototype capillary gradient RFIC system using electrolytic eluent generation and electrochemical detection is shown in Figure 1. In this prototype system, the generation of potassium methanesulfonate and potassium hydroxide eluents is accomplished through the use of a capillary electrolytic KOH generator cartridge and a capillary methanesulfonic acid (MSA) generator cartridge. A high-pressure pump is used to deliver a stream of deionized water into the electrolytic KOH generator cartridge where the high-purity KOH eluent is generated electrolytically. This stream of KOH eluent is then directed into the electrolytic MSA generator cartridge where MSA is generated electrolytically. KOH reacts with MSA to form a solution of potassium methanesulfonate.

As shown in Figure 1, a catalyst column packed with platinum is placed downstream from the electrolytic MSA generator and serves the function to catalytically combine some of hydrogen and oxygen gases formed by the electrolytic operation of the KOH and MSA eluent generators into water. The other downstream system components include a high-pressure degas assembly used for complete removal of hydrogen and oxygen gases, a sample injector, a separation column, and an amperometric detector.

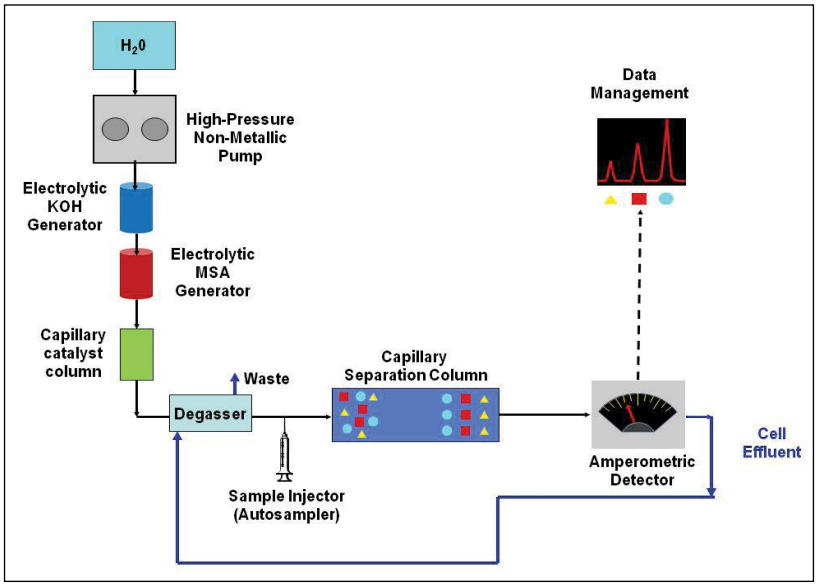

Figure 1. Block diagram of key components in a prototype capillary RFIC system using electrolytic eluent generation and electrochemical detection.

This study evaluates the use of the prototype gradient RFIC system with electrochemical detection for separation of carbohydrates and amino acids. The separation of sialic acids including 27 µM *N*-acetylneuraminic acid (NANA) and 22 µM *N*-glycolylneuraminic acid (NGNA) was performed using a capillary Thermo Scientific™ Dionex™ CarboPac™ PA20 column. The separation of inulin oligomers was performed using a prototype capillary Dionex CarboPac PA200 column. A prototype capillary Thermo Scientific™ Dionex™ AminoPac™ PA10 column was used in the gradient separations of 17 amino acids. Table 1 summarizes the experimental conditions.

Table 1. Typical experimental conditions used in the prototype capillary RFIC system with electrochemical detection.

Parameter	Conditions
Columns	Capillary Dionex CarboPac PA20, 0.4 × 150 mm Prototype Dionex CarboPac PA200, 0.4 × 250 mm Prototype Dionex AminoPac PA10, 0.4 × 250 mm
Temperature	30 °C
Flow Rate	8 µL/min or 10 µL/min
Working Electrode	Au PTFE Disposable Electrode for Carboydrates Au Convention Electrode for Amino Acids
Detection Waveform	Qyadruple potential carbohydrate waveform or *AAA-Direct* waveform
Injection Volume	0.4 µL
Standard Concentration	As indicated in captions to chromatograms

Results and Discussion

The separations of carbohydrates and amino acids on conventional-scale columns in ion chromatography are typically achieved using sodium hydroxide and sodium acetate eluents at varying concentrations delivered by a high-pressure gradient pump fitted with a low-pressure proportioning valve. To explore the benefits of capillary IC for determination of target analytes such as carbohydrates and amino acids, we have carried out preliminary studies on a new platform to perform capillary-scale gradient separations of carbohydrates and amino acids using multi-component eluents generated electrolytically.

The electrolytic eluent generator in the capillary format provides an ideal eluent generation and delivery system at low µL/min flow rates. First, electrolytic eluent generators with small dead volumes can be prepared. Second, electrolytic eluent generators are capable of providing high-fidelity gradient profiles at low µL/min flow rates in a highly reproducible manner through precise current and flow rate controls. Third, it is much more practical and cost-effective to generate higher concentrations of eluents over extended period of time at low µL/min flow rates. For example, the yearly consumption of potassium source ions is 0.263 mol if a capillary Thermo Scientific Dionex EGC-KOH cartridge is used to generate 50 mM KOH at 10 µL/min continuously. On the other hand, 26.3 mol of potassium source ions are needed to generate 50 mM KOH at 1.0 mL/min continuously for one year.

In the system shown, the eluents generated electrolytically using the KOH and MSA generator cartridges may contain KOH, MSA, and KMSA at concentrations depending on the electrical currents applied to the two eluent generator cartridges and the flow rate of the deionized water carrier stream. For example, if the KOH eluent generator cartridge is controlled to generate 150 mM KOH and the MSA eluent generator cartridge is controlled to generate 100 mM MSA, the effluent from the MSA cartridge contains 50 mM KOH and 100 mM KMSA. The concentration of KOH and KMSA can be varied conveniently by varying the electrical currents to the two generator cartridges. Therefore, the embodiment illustrated in Figure 1 provides a novel platform for generating and forming gradients of multi-component eluents using deionized water as the carrier stream. Because of low dead volume associated with the capillary-scale eluent generator cartridges, this novel platform is capable of providing high-fidelity gradient profiles for multi-component eluents at low µL/min flow rates without the need of mechanical proportioning.

Figure 2 shows the separations of sialic acids on a capillary Dionex CarboPac PA20 column at 30 °C and 8 µL/min. Injection volumes of 0.4 µL containing 27 µM NANA and 22 µM NGNA were analyzed. The capillary Dionex EGC-MSA and Dionex EGC-KOH cartridges were controlled to produce constant concentrations of KOH and KMSA eluents. Faster elution of NANA and NGNA was obtained using the eluent contaning 170 mM KOH and 10 mM KMSA.

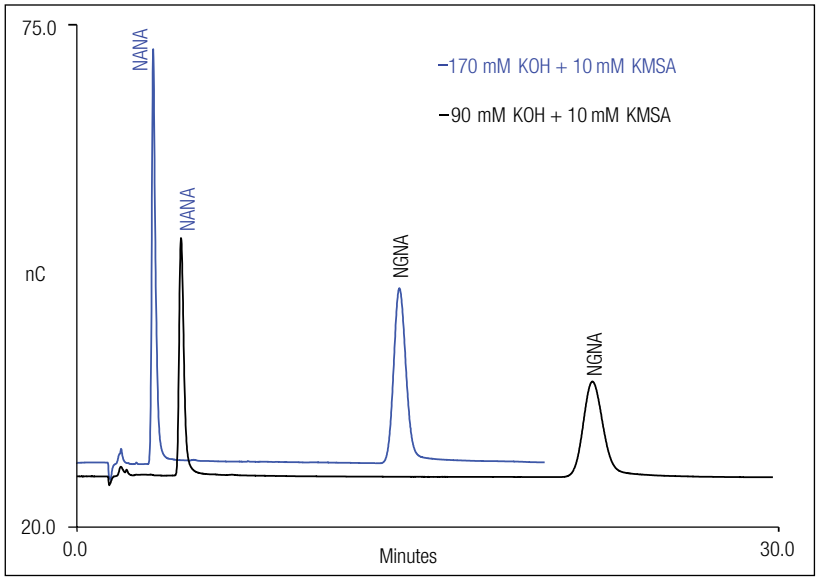

Figure 2. Isocratic separation of sialic acids on a capillary Dionex CarboPac PA20 column.

Figure 3 shows an example of the gradient separation of sialic acids on a capillary Dionex CarboPac PA20 column at 30 °C and 8 µL/min. The separation was obtained for a 0.4 µL sample containing 27 µM NANA and 22 µM NGNA. In this separation, the capillary Dionex EGC-KOH cartridge was controlled to produce 180 mM KOH constantly. In the meantime, the capillary Dionex EGC-MSA cartridge was controlled to produce 10 to 60 mM MSA from 0 to 15 min. Therefore, the composition of the eluent going into the separation column was varied from 170 mM KOH and 10 mM KMSA at the beginning of the gradient run to 120 mM KOH and 60 mM KMSA at 15 min. By using increasing KMSA concentration in the eluent under the gradient condition used, it was possible to obtain fast elution of NANA and NGNA from the capillary Dionex CarboPac PA20 column in less than 10 min.

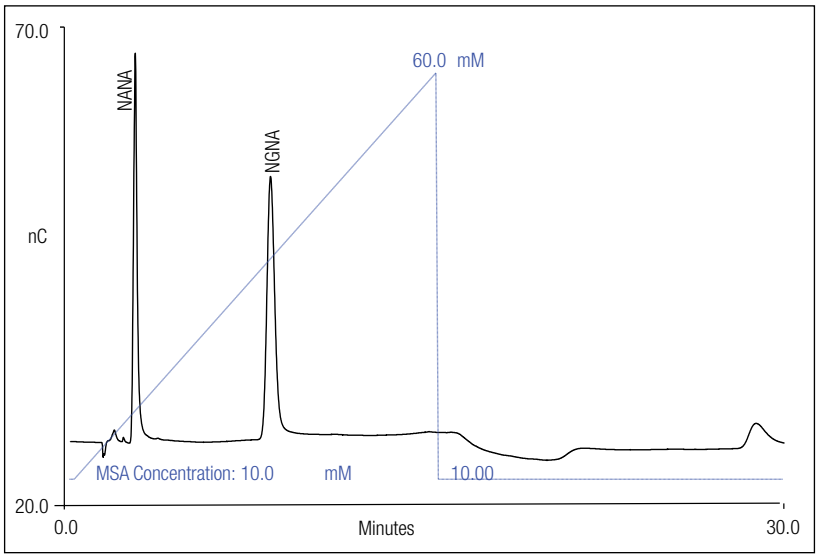

Figure 3. Gradient separation of sialic acids on a capillary Dionex CarboPac PA20 column.

Figure 4 shows the gradient separation of inulin oligomers, which are a group of naturally occurring polysaccharides, on a prototype capillary Dionex CarboPac PA200 column at 30 °C and 10 µL/min. The separation was obtained for a 0.4 µL sample containing 56 mg of inulins in 20 mL 0.1 N NaOH. To achieve the resolution of inulin oligomers, the capillary Dionex EGC-KOH cartridge was controlled to produce 180 mM KOH constantly. In the meantime, the capillary Dionex EGC-MSA cartridge was controlled to produce 55 to 140 mM MSA from 0 to 30 min and 140 mM MSA from 30 to 70 min. Therefore, the composition of the eluent going into the separation column was varied from 125 mM KOH and 55 mM KMSA at the beginning of the run to 40 mM KOH and 140 mM KMSA at 30 min, and maintained at 40 mM KOH and 140 mM KMSA from 30 to 70 min. Under the gradient condition used, excellent resolution of inulin oligomers was achieved using the prototype system.

The gradient separation of hydrolysate amino acids was also obtained using one of the prototype systems in this study. In this case, the capillary Dionex EGC-MSA and EGC-KOH cartridges were pumped with deionized water independently. The KOH and MSA eluents formed were mixed via a tee before being directed into the separation column. The electrolytic eluent generation platform makes it possible to generate rather complex gradient profiles of KOH and KMSA eluents to achieve the separation of 17 hydrolysate amino acids on a prototype capillary Dionex AminoPac PA10 column as shown in Figure 5.

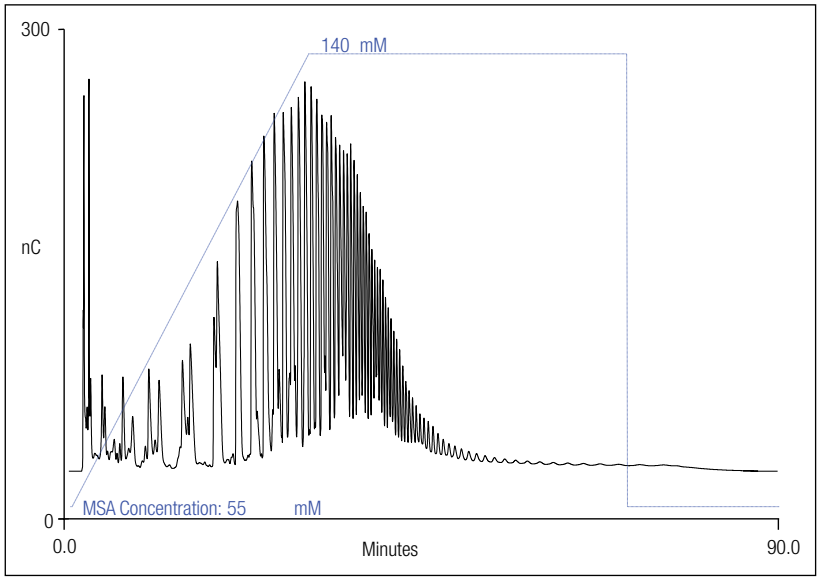

Figure 4. Gradient separation of inulin oligomers on a prototype capillary Dionex CarboPac PA200 column.

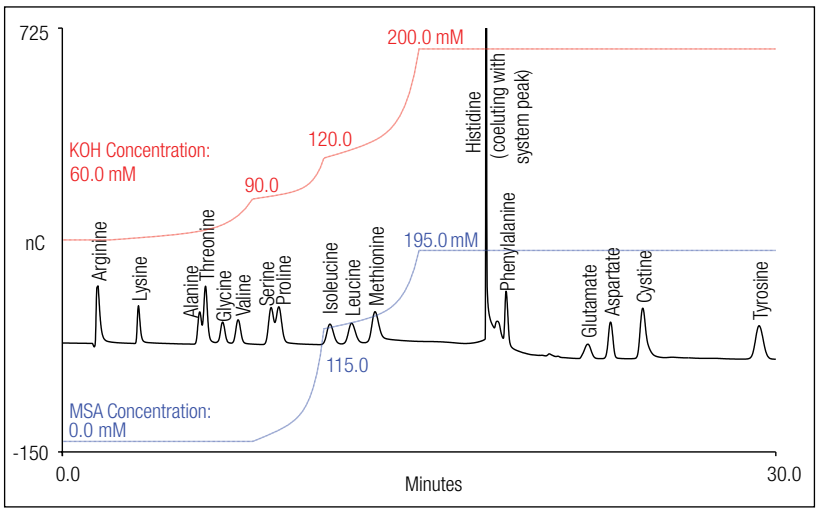

Figure 5. Gradient separation of 17 hydrolysate amino acids on a prototype capillary Dionex AminoPac PA10 column.

Conclusion

Capillary IC systems with on-line electrolytic eluent generation deliver multi-component eluents at low µL/min flow rates and provide high-fidelity gradient profiles for multi-component eluents without the need for mechanical proportioning. These platforms enable highly reproducible gradient separations of carbohydrates and amino acids.

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WP70462_E 08/16S